The PCR products produced by these primers, from a range of medically important Gram positive and Gram negative bacterial cultures, are characterized by hybridization to an array of oligonucleotides designed to identify taxonomic groups. Using this procedure, which takes typically less than four hours, we have been able to identify a wide range of genera and species. This approach allows bacteria and mixtures of bacteria to be identified by molecular methods without the need for a priori knowledge of the causative agent or agents.

The oligonucleotide probes, the sequences of which are set out below, can be used singly for the identification of certain individual species or in a panel or array for the identification of many different species. There is theoretically no limit to the number of oligonucleotide targets employed and the number of species that can be identified.

Ideally the oligonucleotides used should hybridize only to one bacterial species and to all members of that species. Thus with an ideal array, a unique profile consisting of species specific spots would be seen, giving identification to the species level. In practice, two or more oligonucleotide spots may be required for many species and in some cases several such spots may allow identification of variation within a species. In addition, some identifications can be made by comparing the relative intensities of hybridization of individual species to individual oligonucleotides. The assessment of hybridization can be quantified by visual or automated methods.

For example, 27 oligonucleotides have been used for the unambiguous identification of Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus feacium and Enterococcus feacalis , as well as Staphylococcus aureus, coagulase negative Staphylococcus, Listeria species, Stenotrophomonas maltophilia , Burkholderia cepacia, and Escherichia coli. Usually, therefore, it will be desirable to provide oligonucleotides to probe not only for the 8, 10, or more of the micro-organisms commonly occurring in hospital samples or the samples being tested in other situations, but also for other organisms likely to be encountered. Preferably, probes for at least 30 different species of micro-organism will be present on the support substrate used in the test.

CLAIMS

1. A method for identifying bacteria in a sample which comprises amplifying a portion of the 23S rDNA present in the sample using, as one primer, a degenerate primer set comprising one or more DNA molecules consisting essentially of DNA having the sequence(s)

5'GCGATTTCYGAAYGGGGRAACCC

the other primer consisting essentially of DNA having the sequence

5'TTCGCCTTTCCCTCACGGTACT

and testing the resulting amplicon by hybridisation to one or more oligonucleotide probes designed to identify one or more bacteria likely to be present in the sample.

2. Method according to claim 1, in which at least 8 bacterial species are tested for.

3. Method according to claim 2, in which the organisms tested for comprise at least one of Escherichia coli , Staphylococcus aureus , Pseudomonas aeruginosa , Enterococcus spp. , Klebsiella spp. , Enterobacter spp. , Proteus spp, Pneumococci, and coagulase negative Staphylococci.

4. Method according to claim 1, in which at least 10 bacterial species are tested for.

5. Method according to claim 4, in which the organisms tested for comprise at least one of Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus feacium, Enterococcus feacalis , Staphylococcus aureus, coagulase negative Staphylococcus, Listeria species, Stenotrophomonas maltophilia , Burkholderia cepacia, and Escherichia coli.

6. A method according to claim 1, in which the oligonucleotide probe or probes has/have a sequence(s) selected from the group consisting of

SEQ ID Nos 3-7, 9-13, 15-19, 21-28, 30-32, 39-41, 44-49, 51, and 53-58.

7. A method according to claim 1, in which the oligonucleotide probe or probes has/have a sequence(s) selected from the group consisting of SEQ ID Nos 8, 14, 20, 29, 33-38, 42, 43, 50, 52, and 59.

8. A method according to claim 1, in which the oligonucleotide probe or probes has/have a sequence(s) selected from the group consisting of SEQ ID Nos 3-59.

9. A method according to claim 1, in which the oligonucleotide probe or probes has/have a sequence(s) selected from the group consisting of SEQ ID Nos 60 -63.

10. A method according to any of claims 1 to 9, in which amplification is carried out by the polymerase chain reaction (PCR)

11. A method according to any of claims 1 to 9, in which amplification is carried out by transcription mediated amplification.

12. A method according to any of the preceding claims, in which a plurality of oligonucleotide probes are used attached to a support material.

13. A degenerate primer set essentially comprising DNA having the sequences
5'GCGATTTCYGAAYGGGGRAACCC

14. A primer consisting essentially of DNA having the sequence
5'TTCGCCTTTCCCTCACGGTACT

15. A DNA sequence according to claim 13 or 14, being a labelled sequence.

16. A Digoxigenin-labelled DNA sequence according to claim 15,

17. One or more Oligonucleotides consisting essentially of one or more DNA molecules having sequences specified in claim 6.

18. One or more Oligonucleotides consisting essentially of one or more DNA molecules having sequences specified in claim 7.

19. One or more Oligonucleotides consisting essentially of one or more DNA molecules having sequences specified in claim 8.

20. One or more Oligonucleotides consisting essentially of one or more DNA molecules having sequences specified in claim 9.

21. One or more oligonucleotides according to any of claims 17 to 20, immobilised on a solid carrier.

22. A solid support material carrying one or more oligonucleotide probes as specified in claim 6, 7, 8, or 9.

23. A support material according to claim 22, in which some or all of the probes are attached to the substrate by means of chemically modified or additional bases.

24. A support material according to claim 23, in which additional thymine bases have been attached to the 3 prime end of the probe to increase hybridization intensity.

25. A diagnostic kit for the identification of bacteria comprising one or more amplification primers specified in claim 1.

26. A diagnostic kit for the identification of bacteria comprising one or more oligonucleotide probes as specified in claim 6, 7 , 8, or 9.

27. A diagnostic kit for the identification of bacteria comprising a solid support material carrying one or more oligonucleotide probes as specified in claim 6, 7 , 8, or 9.